# Causes of Deterioration of Fats and Oils

## *Exceedingly Small Changes Caused by Oxidation and by Micro-organisms are Sufficient to Produce Detectable Taints*

*By* W. L. DAVIES*

Part II

### *Tests for Auto-oxidation*

THE course of oxidation has been followed by chemical methods. One of these consists in seeking the presence of one type of end product, *e.g.*, an aldehyde, by means of decolorized magenta (Schiff's reagent or the Fellenberg test). The Fellenberg test is a very delicate one, especially when it is carried out on the fat in petroleum ether solution. A slight trace of aldehyde can be detected by a faint coloring (magenta) of the interface between the aqueous and the ethereal layer. In on case has this test been given by a fat before evidence of deterioration was plainly detected organoleptically. On the other hand, it has been found useful for chemically confirming a suspected taint of milk, due to deterioration of the milk fat, the cause of the taint being slight oxidation of the fat at low temperature.

A second method is to take advantage of the color formed by some aldehydes when they combine with phloroglucinol and the presence of hydrochloric acid (*e.g.*, the furfural test). The Kreis test is carried out as follows:—An ethereal solution of the fat is mixed with an ethereal of phloroglucinol and the whole shaken up with an equal volume of concentrated hydrochloric acid. An oxidized fat will give a red coloration of the aqueous layer, the depth of color depending upon the degree of oxidation. The test has been found to be due to the formation of the phloroglucide of epihydrin aldehyde (one of the possible aldehydes from glycerol oxidation). The test can be obtained with a dilute solution of glycerol oxidized with acid bichromate in the same way. Acrylaldehyde will not give the test except in the presence of hydrogen peroxide. This test, again, in no case gave a positive result until the deterioration of the fat was plainly evident to the senses.

A third method lies in the estimation of the "oxidizability values" of the steam-volatile or the water-soluble products of oxidation. This is carried out by obtaining the products, oxidizing them with acid permanganate, and calculating the number of milligrams of oxygen necessary to oxidize the same products from 100 grams of fat. These determinations are, of course, only roughly comparative, and are laborious and cumbersome when the amount of information they offer is considered. The determination of the "oxidizability value" of the water-soluble products is the simpler to carry out, and consists of keeping 25 g. of fat in contact with 100 cc. of water at 80°C. (on a waterbath) for two hours, filtering, oxidizing an aliquot portion with excess of acid N/100 permanganate (boiling for exactly five minutes), and estimating the excess permanganate with N/100 oxalic acid.

These tests have been found useful in following the rates of proper fat oxidation under varying conditions, but have been found valueless in detecting incipient deterioration. As stated above, the search for a variation in constants of the fat is only of use when deterioration has been going on for a considerable time. Such determinations afford a rough indication of what processes have occurred during the prolonged auto-oxidation of the fat.

### *Increase in Acidity*

WITH slow fat deterioration there is a slow increase in acidity. This brings about a state of acid rancidity. This is more marked when auto-oxidation occurs in a fat system catalyzed only by the products of oxidation. If, by some means such as the introduction of

* *Reprinted from the Chemical Age.*

a powerful positive catalyst (or pro-oxygenic agent) such as a trace of metal the oxidation is speeded up, it will be found that the couse of oxidation is turned towards the formation of end-products without the accompanying formation of free fatty acids. This brings about a state of "tallowiness" in the fat, conferring on it not the sour deterioration of fat but a neat's-foot oil odor. (A parallel case occurs with biologically induced rancidity; a pure cuture of a dry mould will give a pleasant ketonic odor at the start, but when other moulds enter this is destroyed by acid formation, replacing the ketonic rancidity by an acid rancidity which is much more nauseous.)

There is thus a difference in effect produced when oxidation only is powerful, as against oxidation coupled with free-fatty-acid formation. This is important in detecting the cause of bleaching and tallowiness in butter. An advanced state of deterioration and discoloration without high acidity invariably points to metallic contamination of the stored butter, whereas high acidity and acid deterioration point to poor storage of the butter, *e.g.,* exposure to too high a temperature and to light, and high initial acidity of the product. Micro-estimations of copper and iron in such butters have borne out this view.

### *Tests for Keeping Quality*

THE factors which catalyze oxidation proper in a fat also shorten the induction period. Therefore, in a sample of fat which will have a short life in the wholesome state, there is a factor (which possibly could be detected by a method using an external indicator) which is as yet latent but which will in time show its presence both in shortening the induction period and in catalyzing the oxidation proper. The main properties of this agent or factor lie in the fact that it can carry oxygen to the double bond of the oleic acid residue, thus initiating the oxidation, and this formation of peroxide takes place to a small extent when any fat is exposed to the air. The amount of peroxide formed is usually too small to be determined quantitatively, but its presence can easily be demonstrated by the usual peroxide tests, *e.g.,* the blueing of guaiacum and the indophenol test.

An attempt has been made to demonstrate the varying tendency to oxidation (owing to the small amounts of catalyst present) by studying the degree to which leuco-methylene blue can be oxidized by atmospheric oxygen through the agency of these catalysts. In testing a series of fats, the fat, emulsified in fat-free milk, is reduced (free of peroxides) along with dilute methylene blue by the reductase of the milk flora. On shaking the white liquids after reduction is complete (as evidenced by a state of total bleach) with air for a definite time and allowing a definite time of standing (usually two minutes), a variation in the degree of restoration of the blue color can be observed. This degree of restoration varies directly with the keeping quality of the fat—the deeper the blue color restored, the shorter time will the fat under consideration remain in the wholesome state. This test has been found useful in detecting traces of metals which in a short time will cause tallowiness in fats (*e.g.,* butter). Further work on the oxidation potentials of fat emulsions should prove useful in this direction.

High temperatures hasten the progress of fat oxidation enormously. Hence, by working at high temperatures, it should be possible to obtain information as to the relative lengths of the periods of induction for a series of fats. This could be accomplished: (*a*) By determining the lengths of the periods of induction of the fats at 90° C., using an oxygen uptake apparatus, which could be fitted with a kymograph registering mercury levels in the free manometer tube at atmospheric pressure; (*b*) Samples of fats could be exposed at 90° C. in open dishes, the time of heating necessary for the sample to give either a positive Fellenberg or a Kreis test being noted. These tests are used considerably in determining the keeping qualities of samples of lard. Despite much investigation, our resources for grading fats according to keeping quality are still small.

### *Biological Deterioration*

FATS are of biological origin. Their non-aqueous nature naturally precludes them from being suitable media for the growth of micro-organisms. Nevertheless, micro-organic contamination might modify the usual process in the contaminating organism so as to cause sufficient breakdown of the fat molecule to be detectable by taste and smell.

In the growth of any organism a source of energy is an all-important factor. An aerobic organism gets its energy by burning organic matter during the process of respiration. If that respiration is impeded, then some organisms will derive their energy by fermentative processes. An organism contaminating an oil or fat will have its cell wall coated with a layer of oil or fatty acids and its respiration will be modified. It will then seek energy anaerobically, namely, by attacking some of the glycerine of the fat or fatty acids. The liberation of glycerine needs an enzyme, "lipase," which is present in variable amounts

in various organisms. A variation in effect on fats, therefore, is to be expected from different organisms, and this is actually the case.

It must be pointed out at the outset that the decomposition of fats by micro-organisms is of a different nature from that described above for the auto-catalytic oxidation of fats( called chemical deterioration). Also it is interesting to note that if micro-organisms are present in fats, no auto-catalytic oxidation will occur. Any available oxygen in the fat will be used up by the micro-organisms. This can be shown by suppressing the proliferation of micro-organisms in an already contaminated fat, *e.g.,* either by cold storage or other means, and examining the nature of subsequent change. In all cases auto-oxidation will occur, and tallowiness will gradually set in. This is the main cause of the totally unexpected deterioration of cold stored butter. Whereas it was thought that depression of micro-organic growth would save the situation with respect to butter storage, the conditions resorted to immediately favored an equally disastrous change, especially when auto-oxidation was so realily catalyzed by the minute traces of heavy metals which had entered the material during processing.

The same remark applies to milk. The souring of milk causes the greatest loss in the trade, and this has been overcome by endeavoring to keep the milk at as low a temperature as possible during transportation and storage. The fat in milk presents an enormous surface for oxidation, and in such cases, where traces of copper have entered the milk during treatment (*e.g.,* from copper coolers), a taint termed "oiliness" develops on occasion when such milk is kept in the cold. The depression of bacterial growth by ultra-violet radiation must for this reason be carried out with care. The activation of dissolved oxygen soon makes its effect apparent on the milk fat. There would be less risk if the flora of the milk had had an opportunity of consuming the dissolved oxygen before irradiation.

### *The Effect of "Foots"*

THE purity of a fat or oil has a marked effect on its keeping quality. Generally, for good keeping quality, an oil should have a low free-organic-acid content and complete absence of extraneous aqueous material such as "foots." Where vegetable oils are extracted from the seed, a certain amount of protein material finds its way into the crude extract. This protein material is usually rich in a fat-splitting enzyme (lipase) derived from the embryo, and this enzyme was originally intended for breaking up the seed fat to supply energy to the growing embryo. In the presence of "foots," therefore, a certain amount of fat-splitting will occur, and there will be a rise in acid-value, which will be detrimental to the keeping quality of the fat when subsequently separated from the "foots." The use of damaged seed for oil crushing has the same effect. This fat-splitting is the more evident in commerce with the cereal oils and olive oil.

### *Bacteria: Surface Tainting*

THE activity of any organism in a medium depends on the use it can make of the substrate. With fat as medium, part of the resultant activity will depend on the accommodative power of the organism to utilize the substance at its disposal under the existing conditions. On bacteria, fat will generally exert a modified bacterio-static effect by coating the cell wall with a non-aqueous layer of material. Other factors governing the welfare of the organism are its lipolytic activity, the toxicity of products of lipolysis (*e.g.,* the fatty acids) and its power of dealing with these products; the presence of foreign material in the fat, such as proteins and soluble organic acids (lactic and succinic); the amount of water; and its proximity to atmospheric oxygen. These factors govern the welfare of any micro-organism, whether aerobic, anaerobic, or micro-aerophyllic.

Comparatively speaking, deterioration of fat due to bacteria is not so marked as that due to higher organisms, such as moulds, largely owing to the fact that they have not the same property of extending mycelium or extruding aerial fructifications, neither have they the high accommodative powers of the moulds. The worst cases of deterioration occur where atmospheric oxygen can reach the organisms. This gives rise to the phenomenon of surface tainting, where fat in contact with atmospheric oxygen is broken down to yield products which are repulsive to taste and smell. The general change is that of methyl-ketone formation, as will be described below for moulds. At the surface there may also be detected a change in acid value. That the organisms are respiring can be detected by aerating off the carbon dioxide formed over a lengthy period. In their slow action on fats, it seems noteworthy that the organisms have a selective power as to the isomeric form of unsaturated acid which they will attack, and this is invariably the cis-form. Oleic acid is readily attacked, while its isomer, elaidic acid, is not. The destruction of bacteria in fats by sterilization is a questionable process, as some organisms are heat-resistant when coated with a layer of fatty acid or calcium soap. Taints in sterilized milk are attributed to this fact.

*Moulds*

MOULDS are the organisms which cause most deterioration in fats and oils, since they are less specific and more accommodative than bacteria. Their action on fats, also can be much more readily followed. Moulds, in their action on fats, are divided into two classes: (*a*) Those which preserve the solid consistency of the butter; and (*b*) Those which have strong lipolytic properties and liberate much fatty acid, mostly oleic acid, thus rendering the fat oily.

To the first class, the higher fatty acids in a free state are toxic, and consequently, the lipase content of these organisms is not high. On the other hand, the lower fatty acids, from capric acid downwards, are not toxic, and these are acted on; the effect of these "dry" moulds on fats containing appreciable quantities of these acids, *e.g.*, butter-fat and coconut oil, is interesting. The process consists of $\beta$-oxidation of the acid to form first the secondary alcohol and then the methyl ketone containing one carbon atom less:

$$CH_3(CH_2)_n\ CH_2CH_2COOH \rightarrow$$
$$CH_3(CH_2)_n\ CHOHCH_2COOH \rightarrow$$
$$CH_3(CH_2)_nCOCH_3 + CO_2.$$

Thus caproic, caprylic and capric acids liberated from the fat by lipolysis are changed into methyl amyl, methyl heptyl, and methyl nonyl ketones respectively. In small amounts, these ketones have the odor of coconut, but in larger quantities, their presence becomes repugnant to taste and smell. There is very little increase in acidity with the development of the taint, and oleic acid present in the free state has a retarding effect on the growth of the above moulds in fats. Following through the changes in the Reichert-Meissl and Polenske values of the fats when acted upon thus, it is quite easy to see what acids are being broken down. Among the active moulds are *Penicillium, Aspergillus,* and *Cladosporium.*

The second class of moulds, the acid-formers, act in a different way. They have a high lipase content, and the higher fatty acids do not poison their mycelium. They break the acids down, again by $\beta$-oxidation, to lower fatty acids, each losing two carbon atoms in each step of breakdown. Oleic acid is formed in large quantities in such cases, giving the fat a lower solidifying point and the free fatty acids a high iodine value. The capacity of these moulds to oxidize the acids is much stronger than with the previous class. Lipoids in the fats are also oxidized, usually before the fat proper is greatly acted upon. *Oidium* and *Oospora* are examples of this type of mould. These can break down butter lecithin to give fishy butter, whereas the "dry" moulds break down the curd to give a cheesy taint to the butter. The breakdown of the fatty acids occurs simultaneously for all of them, as there is no predominance of one class of fatty acid mounting up during prolonged activity of the mould, except oleic acid, which is the result of vigorous lipolysis of the olein. This acid, in the free state, may be used as an oxygen carrier for the moulds.

Very little is known of the effect of yeasts on fats. A distinct ester smell (ethyl butyrate) may be generated in milk from lactose fermentation and lipolysis of butyrin of butter-fat, but such cases have not been noted in butter-fat or butter. The relation of temperature of storage and micro-organic activity to the process of fat deterioration is of importance. Where the temperature favors micro-organic growth, auto-oxidation is precluded, while at low temperatures, where micro-organic growth is depressed, auto-oxidation will proceed. The remedy, therefore, is to refine the fat in such a way that the amounts of mould and of catalysts for auto-oxidation are at a minimum in the product. Butter is a striking example of this. The more the raw material is processed, the more will metal of a deleterious nature enter it. Pasteurization overcame some of the faults, only to show that on cold storage favorable conditions for auto-oxidation obtained.

---

A study of the chemical equilibrium during fat splitting and fat saponification, applying the mass $K = (G)(S)^3/(F)(W)^3$, in which G is glycerol, S fatty acids or soap, F is fat and W is water or caustic soda, shows that the highest degree of splitting, after equilibrium is reached, depends upon the temperature and composition of the fat and not upon the type of process employed. It also shows that under the same conditions of reaction, the percentage of splitting is greater, the greater the original amount of water present. The constant K in relation to temperature is found from the formula lok $K = (q/4.573T) + c$, where q is heat of reaction, T is absolute temperature, c is a constant, and 4.573 is the product of the gas constant and the conversion factor of natural logarithms into ordinary logarithms. *Allegem. Ol-Fettztg.* 27,114-5 (1930). *Chem. Abstr.* 24,494-6 (1930).

---

Wilson & Bennett Mfg. Co., manufacturers of steel containers, Chicago. are now constructing a new three-story office addition to their factory.